

Locomotor and diaphragm muscle fatigue in endurance athletes performing time-trials of different durations

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Abstract

Purpose Fatigue in leg muscles might differ between running and cycling due to inherent differences in muscle activation patterns. Moreover, postural demand placed upon the diaphragm during running could augment the development of diaphragm fatigue.

Methods We investigated quadriceps and diaphragm fatigue in 11 runners and 11 cyclists (age: 29 ± 5 years; $\dot{V}O_{2,\text{peak}}$: 66.9 ± 5.5 ml min⁻¹ kg⁻¹) by assessing quadriceps twitch force (Q_{tw}) and transdiaphragmatic twitch pressure ($P_{\text{di,tw}}$) before and after 15- and 30-min time-trials (15TT, 30TT). Inspiratory muscle fatigue was also obtained after volitional normocapnic hyperpnoea (NH) where postural demand is negligible. We hypothesized that running and cycling would induce different patterns of fatigue and that runners would develop less respiratory muscle fatigue when performing NH.

Results The reduction in Q_{tw} was greater in cyclists (32 ± 6 %) compared to runners (13 ± 8 %, $p < 0.01$), but not different for 15TTs (23 ± 13 %) and 30TTs (21 ± 11 %, $p = 0.34$). Overall $P_{\text{di,tw}}$ was more reduced after 15TTs (24 ± 8 %) than after 30TTs (20 ± 9 %, $p = 0.04$) while being similar for runners and cyclists ($p = 0.78$). Meanwhile, breathing duration in NH and the

magnitude of inspiratory muscle fatigue were also not different (both $p > 0.05$).

Conclusion Different levels of leg muscle fatigue in runners and cyclists could in part be related to the specific muscle activation patterns including concentric contractions in both modalities but eccentric contractions in runners only. Diaphragm fatigue likely resulted from the large ventilatory load which is characteristic for both exercise modalities and which was higher in 15TTs than in 30TTs ($+27$ %, $p < 0.01$) while postural demand appears to be of less importance.

Keywords Self-paced exercise · Fatigue · Locomotor muscles · Respiratory muscles · Hyperpnoea

Abbreviations

CV	Coefficient of variation
MVV	Maximal voluntary ventilation
M-wave	Compound muscle action potential
P_{di}	Transdiaphragmatic pressure
$P_{\text{di,tw}}$	Transdiaphragmatic twitch pressure
P_{es}	Esophageal pressure
$P_{\text{es,tw}}$	Esophageal twitch pressure
P_{ga}	Gastric pressure
$P_{\text{ga,tw}}$	Gastric twitch pressure
P_{m}	Mouth pressure
$P_{\text{m,tw}}$	Mouth twitch pressure
$\text{PTP}_{\text{di,in}}$	Inspiratory transdiaphragmatic pressure–time product
$\text{PTP}_{\text{ga,in}}$	Inspiratory transdiaphragmatic pressure–time product
$\text{PTP}_{\text{es,in}}$	Inspiratory esophageal pressure–time product
$\text{PTP}_{\text{es,ex}}$	Expiratory esophageal pressure–time product
$\text{PTP}_{\text{ga,ex}}$	Expiratory gastric pressure–time product
Q_{tw}	Quadriceps twitch force

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$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2,\text{peak}}$	Oxygen consumption at peak workload
WOB	Work of breathing
$\Delta P_{\text{di,tw}}$	Reduction in transdiaphragmatic twitch pressure
ΔQ_{tw}	Reduction in quadriceps twitch force
15TT	15 min time-trial
30TT	30 min time-trial

Introduction

Neuromuscular fatigue develops when one or several processes which allow the muscle to contract and thus to generate force are impaired, independent of whether a given task can be sustained or not (Bigland-Ritchie and Woods 1984). These impairments can occur at different sites between brain and muscle and the location and extent strongly depend on the task that is performed (Allen et al. 2008; Gandevia 2001). How different components of fatigue contribute to exercise limitation has been a matter of extensive debate and several different models were introduced (Abbiss and Laursen 2005). Experimental data suggest that the level of peripheral locomotor muscle fatigue associated with unconscious and/or consciously perceived afferent feedback might contribute to these exercise limiting factors (Amann 2011). Amann and co-workers (2009) showed that attenuation of type 3 and 4 afferent feedback can substantially alter central motor output to working limbs in cycling exercise resulting in unusually high levels of peripheral fatigue. It is thus believed that type 3 and 4 afferent feedback from working muscles likely contributes to the impaired willingness and/or ability to drive a muscle in order to avoid the development of fatigue beyond a certain functional limit (Amann and Secher 2010).

To date, several studies have described the occurrence of locomotor muscle fatigue in knee extensors and plantar flexors during self-paced running or cycling exercise with test durations and intensities ranging from brief sprints up to events of several hours and days (Martin et al. 2010; Millet et al. 2011; Ross et al. 2010; Saugy et al. 2013; Skof and Strojnik 2006a, b). A broad range of reductions (i.e. 0–30 %) in muscle twitch force of these muscles in response to nerve stimulation have been reported. Yet, no study has systematically investigated the extent of leg muscle fatigue in self-paced exercise of different durations and intensities performed by the same individuals. Similar end-exercise levels of leg muscle fatigue in such trials would potentially provide further support to the importance of afferent feedback in the regulation of exercise intensity as described above. However, fatigue could also be affected by the type of muscle contraction depending on the exercise modality being performed. Running and cycling—the two most common types of locomotion—are very dissimilar in

terms of the activation pattern (i.e. concentric-eccentric vs. mainly concentric) which suggests different fatigue mechanisms to be present (Bijker et al. 2002).

During intense whole-body exercise not only leg muscles are active and develop fatigue, but also respiratory muscles are under severe strain, the level mainly depending on exercise intensity and the related level of ventilation [e.g. (Johnson et al. 1993; Verges et al. 2006b)]. Respiratory muscle work has been shown to require up to 15 % of whole-body oxygen consumption ($\dot{V}O_2$) at peak workload ($\dot{V}O_{2,\text{peak}}$) in endurance-trained athletes highlighting the substantial work performed by these muscles (Aaron et al. 1992). In contrast to locomotor muscles, however, fatigue of respiratory muscles has not yet been described for competition like self-paced exercise although fatigue was repeatedly found to develop during intense constant-load exercise [e.g. (Johnson et al. 1993; Mador et al. 1993)]. Moreover, no data are available using objective measurements to obtain potential differences in the level of stress and thus fatigue of respiratory muscles between running and cycling. The fact that the diaphragm appears to be substantially involved in the maintenance of posture suggests that the load placed on this muscle could be larger in running compared to cycling [e.g. (Hodges and Gandevia 2000)].

Therefore, the aim of the present study was (i) to objectively quantify both locomotor and inspiratory muscle fatigue after competition-like running and cycling time-trials of different durations/intensities (i.e. 15 and 30 min; 15TT and 30TT) in a group of well-trained runners and cyclists, (ii) to investigate potential differences between running and cycling in the level of quadriceps and inspiratory muscle fatigue in these trials, and (iii) to test whether runners and cyclists differ in terms of fatigability during volitional normocapnic hyperpnoea where no additional postural demand from the gait is expected. We hypothesized that both the quadriceps and inspiratory muscles would yield a very specific fatigue profile for running and cycling and that runners would sustain normocapnic hyperpnoea at the same relative intensity for a longer duration than cyclists.

Methods

Subjects

Twenty-two male endurance athletes (runners $n = 11$, cyclists $n = 11$) with normal lung function and respiratory muscle strength (Table 1) gave their written informed consent to participate in this study. Subjects were free of acute or chronic disease and were non-smokers. A minimal $\dot{V}O_{2,\text{peak}}$ of $55 \text{ ml min}^{-1} \text{ kg}^{-1}$, regular physical training, and

Table 1 Subject characteristics for runners and cyclists

	Runners	Cyclists
Age (years)	31 ± 4	27 ± 6*
Height (m)	1.81 ± 0.08	1.81 ± 0.04
Body mass (kg)	72.1 ± 7.3	75.5 ± 8.2
$\dot{V}O_{2,peak}$ (l min ⁻¹ kg ⁻¹)	66.7 ± 5.5	67.2 ± 6.6
MIP (cmH ₂ O)	133 ± 17	138 ± 28
MIP (%pred)	121 ± 16	121 ± 27
MEP (cmH ₂ O)	207 ± 45	208 ± 36
MEP (%pred)	137 ± 31	134 ± 26
FVC (l)	6.20 ± 0.55	6.17 ± 0.81
FVC (%pred)	118 ± 9	114 ± 10
FEV ₁ (l)	4.76 ± 0.34	4.85 ± 0.62
FEV ₁ (%pred)	109 ± 10	107 ± 10
PEF (l s ⁻¹)	10.5 ± 0.8	10.1 ± 0.4
PEF (%pred)	106 ± 8	103 ± 10
MVV (l min ⁻¹)	204 ± 19	197 ± 23
MVV (%pred)	134 ± 13	126 ± 15

Values are mean ± SD. $\dot{V}O_{2,peak}$ peak oxygen consumption, MIP maximal inspiratory pressure, MEP maximal expiratory pressure, FVC forced vital capacity, FEV₁ forced expiratory volume in the first second, PEF peak expiratory flow, MVV maximal voluntary ventilation

* Significantly different between runners and cyclists, $p < 0.05$

frequent participation in endurance competitions (in the respective sports activity) were mandatory to be included in this study. Subjects were requested to keep their personal training constant throughout the course of the study. They were asked to refrain from strenuous physical activity for the 2 days prior to the test, to completely refrain from exercising for 24 h before testing, to sleep at least 7 h the night before the test, to abstain from caffeinated beverages on test days and to have their last meal at least 2 h prior to reporting to the laboratory. The study was approved by the local ethics committee and was performed according to the Declaration of Helsinki.

Experimental overview

Subjects reported to the laboratory on four different days separated by at least 48 h and scheduled at the same time of day to control for confounding circadian influences (Scheer et al. 2010). All testing had to be completed within a maximum of 3 weeks. Runners performed all tests on a treadmill while cyclists performed tests either on a stationary cycling ergometer or a road bike mounted on an indoor cycle trainer (for details see below). On the first day, subjects performed an incremental running or cycling test to exhaustion. They were then introduced to pulmonary function and respiratory strength testing and were extensively

familiarized with the technique of magnetic stimulation. On the second day, baseline pulmonary function and respiratory strength measurements were obtained and inspiratory muscle contractility was assessed before and after a bout of exhaustive normocapnic hyperpnoea. On the third and fourth days, diaphragmatic and quadriceps muscle contractility were assessed before and after self-paced running or cycling time-trials of 15 (15TT) and 30 min (30TT) duration, performed in a randomized and balanced order.

Lung function and respiratory muscle strength

Lung function including maximal voluntary ventilation in 12 s (MVV) was assessed according to current ATS/ERS guidelines (Miller et al. 2005) using a metabolic cart with a calibrated volume sensor (Oxycon pro, Jaeger, Höchberg, Germany). Maximal inspiratory pressure (at residual volume) and maximal expiratory pressure (at total lung capacity) were measured using a handheld device (MicroRPM, MicroMedical, Kent, UK). Variables of pulmonary function and respiratory muscle strength are reported both in absolute values and in percent of predicted values (Quanjer et al. 1993; Wilson et al. 1984).

Perception of respiratory and leg exertion

Respiratory exertion and leg exertion were assessed by means of a linear scale ranging from 0 to 10, where 0 corresponded to no and 10 to maximal perception of respiratory or leg exertion. To ensure a proper understanding of the term respiratory exertion, subjects were extensively questioned about their prior experience with respiratory sensations before the first exercise test (Lansing et al. 2000). Thereafter, a definition was given for respiratory exertion (how difficult it is to breathe) which is distinguished from breathlessness (the sensation of “not getting enough air”).

Incremental exercise

An incremental running or cycling test to exhaustion was performed to determine $\dot{V}O_{2,peak}$ as well as maximal running velocity or maximal cycling power output.

For the running incremental test, subjects stood for 5 min on a treadmill (Quasar, h/p/cosmos, Traunstein, Germany) with a mouthpiece and nose clip in place before starting to run with a velocity of 10 km h⁻¹. Thereafter, treadmill speed was increased by 1.5 km h⁻¹ every 2 min to the point of exhaustion. At the end of each stage and at the point of exhaustion subjects were asked to rate their perception of respiratory and leg exertion. Ventilation and gas exchange were measured breath by breath via the metabolic cart with calibrated volume, CO₂ (infrared absorption principle) and O₂ sensors (paramagnetic principle). Heart

rate was obtained beat by beat using a Suunto T6 heart rate monitor (Suunto Oy, Vantaa, Finland). At rest and at termination of exercise, 20 μ l of capillary blood was drawn from an ear lobe to assess blood lactate concentration enzymatically (BIOSEN C_line Sport®, EKF-diagnostic, Barleben, Germany).

For cyclists, the incremental test was performed on a cycling ergometer (Ergoline 900, Ergoline, Blitz, Germany). Cyclists were monitored in the same way as runners. The protocol consisted of a resting period (5 min) before cycling at 100 W for 2 min, followed by increments of 30 W every 2 min until the point of exhaustion. Cycling cadence was chosen by the subject during the first stage and held constant for the remainder of the test. The test was either terminated when subjects voluntarily stopped or when cadence dropped below 70 rpm. In addition to the above-described measurements, samples of capillary blood were drawn at the end of each stage.

Normocapnic hyperpnoea

First, subjects performed 2 min of normocapnic hyperpnoea at 40 % and 2 min at 60 % of their individual MVV to warm up before they were required to breathe at the target ventilation of 80 % MVV to exhaustion. The mouthpiece was connected to a two-way valve (Hans Rudolph, Shawnee, KS, USA) via the volume-sensor of the metabolic cart. Normocapnia was maintained by adding CO₂ to the inspire. Subjects received both visual and verbal feedback to keep minute ventilation at the target level. Visual feedback was provided via a computer screen showing minute ventilation online while verbal feedback consisted of telling the subjects to breathe “more” or “less” (without referring to tidal volume or breathing frequency) when they deviated from the target by ≥ 5 % for several breaths. The test was terminated if target ventilation could no longer be achieved. Before and immediately after normocapnic hyperpnoea, mouth twitch pressure ($P_{m,tw}$) was assessed (for details see below). If $P_{m,tw}$ was reduced by <20 % from baseline, subjects were required to continue breathing at the same target ventilation until task failure was reached again (one subject only).

Time-trial exercise

For running and cycling time-trial exercise, subjects were asked to cover as much distance as possible once in 15 min and once in 30 min.

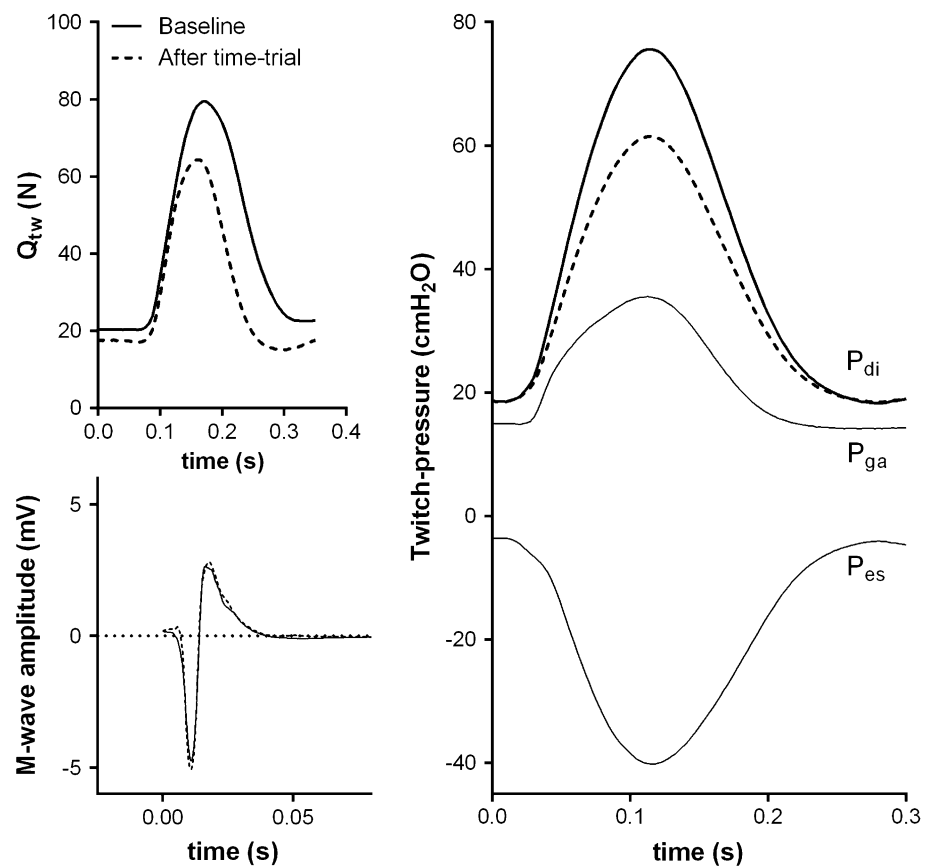
After 2 min at rest subjects were requested to warm up for 5 min at an intensity corresponding to 50 % of their individual maximal running velocity (for runners) and 1.5 W kg⁻¹ body mass (for cyclists) followed by a “running start”, meaning that treadmill speed was steadily increased

during the final minute of the warm up period until subjects signaled that their initial running velocity for the time-trial was reached (within 30–45 s). Cyclists performed on a road bike equipped with a calibrated SRM system (Power MTB, Schoberer Rad Messtechnik SRM, Jülich, Germany) stationed on an indoor cycle-trainer (Tacx Basic Cycleforce, Tacx, Wassenaar, Nederland) and they increased power output to their starting time-trial load approximately 10 s before the start. Thereafter, subjects were allowed to freely adjust their running velocity or cycling power output. All subjects were encouraged verbally throughout the entire test and received feedback of the elapsed time from a screen in front of the treadmill or cycle but were naïve to velocity/power output and distance covered. Ventilation, gas exchange, esophageal (P_{es}) and gastric (P_{ga}) pressures, and heart rate were measured continuously and stored simultaneously in a computer (MacLab Software, ADInstruments, Castle Hill, Australia). At rest, every 3 min during the time-trial as well as at the end of the test, subjects were asked to rate their perception of respiratory and leg exertion. Before and immediately after the time-trial, \dot{Q}_{tw} and $P_{di,tw}$ were assessed (for details see below). At rest and at termination of the time-trial, 20 μ l of capillary blood was drawn from an earlobe to assess blood lactate concentration. In cycling time-trials, additional samples of capillary blood were drawn every 3 min during the test.

Magnetic stimulation of femoral and phrenic nerve roots

Quadriceps muscle contractility was objectively determined by assessing quadriceps twitch torque (\dot{Q}_{tw}) in response to magnetic femoral nerve stimulation before and 10 min after completion of the time-trial (Fig. 1). Subjects were studied semi-supine on a custom-made chair. Care was taken that the knee was flexed at exactly 90° and the leg was passively stabilized to prevent lateral motion. The ankle was fixed to a force transducer (strain gage LC4102-K060, A&D CO, Tokyo, Japan) by a non-elastic ankle strap. Force signals were recorded on the computer. The femoral nerve was stimulated with a 70-mm figure-of-eight coil powered by a MagStim 200 stimulator (MagStim, Whitland, UK). The center of the coil was placed in the femoral triangle just lateral to the femoral artery and was repositioned systematically to determine the position that resulted in the largest \dot{Q}_{tw} . This position was marked and used for the remainder of the study. Sarcolemmal membrane excitability for the quadriceps was determined by means of peak-to-peak amplitudes of compound muscle action potentials (M-wave). M-waves were recorded using bipolar surface silver electrodes on the muscle belly of the vastus lateralis with a recording diameter of 1 cm and an inter-electrode distance of 2 cm. The skin was carefully shaved, treated with an abrasive paste, and cleaned using alcohol prior to

Fig. 1 Response to femoral (left panels) and cervical (right panel) magnetic stimulation before and after a time-trial in a representative participant. Q_{tw} , quadriceps twitch force; M-wave, compound muscle action potential of the quadriceps; P_{es} , esophageal pressure; P_{ga} , gastric pressure; P_{di} , transdiaphragmatic pressure ($P_{di} = P_{ga} - P_{es}$)



electrode placement. EMG signals were recorded at a sampling rate of 4,000 Hz, pre-amplified (gain = 1,000), band-pass filtered (20–1,000 Hz; Nihon Kohden, Bad Homburg, Germany; common mode rejection ratio ≥ 94 dB), A/D converted (MacLab interface, ADInstruments, Castle Hill, Australia), and stored in the computer.

Inspiratory muscle contractility was assessed by means of phrenic nerve stimulation using a circular 90 mm coil powered by the magnetic stimulator before and 2 min after completion of the time-trial (Fig. 1). For assessment of $P_{m,tw}$, a differential pressure transducer (DP45-34, Validyne, Northridge, CA, USA) was connected to a mouthpiece. For assessment of transdiaphragmatic twitch pressure ($P_{di,tw}$), conventional balloon-catheters (esophageal and gastric balloons containing 1 and 2 ml of air, respectively) were inserted through the nose and positioned in the esophagus and in the stomach to measure P_{es} and P_{ga} according to current guidelines (ATS/ERS 2002). Balloon-catheters were separately connected to differential pressure transducers (DP45-34, Validyne, Northridge, CA, USA). Pressure signals were amplified (CD 19A, Validyne, Northridge, CA, USA), A/D converted and recorded in the computer. P_{di} was calculated by online subtraction of P_{es} from P_{ga} . Cervical magnetic stimulation of the phrenic nerves was applied while subjects were comfortably seated on a

chair with a nose-clip in place and the center of the coil was positioned at the 7th cervical vertebra. The subject's position on the chair and the coil position on the neck were marked and continuously monitored throughout the experiment. Stimulations for the assessment of $P_{m,tw}$ during the 2nd visit were performed at the start of a gentle expiratory effort from functional residual capacity through a small leak while stimulations for the assessment of $P_{di,tw}$ were applied at the end of a passive expiration corresponding to functional residual capacity with subjects' mouth closed. P_{es} was continuously monitored on an oscilloscope (Tektronix, Beaverton, OR, USA) by a second experimenter to ensure that identical lung volumes were achieved immediately prior to stimulations. In addition, pre-twitch P_{es} -recordings showing a deviation from baseline P_{es} were rejected post hoc. For one subject, P_{di} could not be calculated due to a defective gastric balloon.

The experimental protocol for the assessment of both inspiratory and quadriceps muscle strength consisted of 9 potentiated twitches at 100 % of the stimulator output. Q_{tw} force was measured in a fully relaxed state. To investigate fatigue-induced changes in muscle contractility, potentiated twitches were used since they are known to be more sensitive to changes occurring within a fatigued muscle (Kufel et al. 2002). Special care was taken to assure that

investigated muscles were indeed in a fully potentiated state. Potentiated twitches were assessed after 3–4 submaximal warm-up contractions and 3 maximal efforts lasting 5 s. After the third and sixth stimulation, another maximal effort followed to maintain the potentiated state. To ensure supramaximal stimulation before and after exercise, 3 additional twitches were performed at 70, 80, 90, 94 and 98 % (for phrenic nerve stimulation) or 60, 70, 80, and 90 % (for femoral nerve stimulation) of the stimulator output either on the third or fourth visit in a randomized and balanced order. A plateau for Q_{tw} and $P_{di,tw}$ was observed in most subjects. Moreover, no statistically relevant difference was found between the highest and the second highest stimulator output before and after exercise indicating maximal depolarization of the femoral and phrenic nerves at all times.

Data analysis and statistics

Lung function variables were determined according to ATS/ERS guidelines (Miller et al. 2005). Maximal inspiratory and expiratory pressures were selected as the highest of 3 values with no more than 5 % deviation. $\dot{V}O_{2,peak}$ was calculated as the highest 15-s average while maximal running velocity and maximal cycling power output (last completed stage + time on the final stage \times increment). For twitch pressures and force, the average amplitude (baseline to peak) of 10 unpotentiated and 6–9 potentiated twitches was calculated. A twitch was rejected post hoc if P_{es} prior to twitch stimulation deviated from baseline. Average peak-to-peak amplitude of M-waves was calculated from valid Q_{tw} responses only. For maximal voluntary diaphragm contractions and maximal voluntary quadriceps contraction the average of the two highest volitional values obtained during the potentiation maneuvers was calculated. Voluntary activation ratio for the diaphragm and the quadriceps was calculated with the following formula:

Voluntary activation ratio (%)

$$= \left(1 - A \times \frac{\text{superimposed twitch amplitude}}{\text{Amplitude of potentiated twitch}} \right) \times 100$$

A correction term A was included to account for superimposed stimulations which were not delivered at the highest volitionally produced pressure/force.

$$A = \left(\frac{\text{Volitional pressure/force just before superimposed twitch}}{\text{Highest volitional pressure/force during maneuver}} \right)$$

Ventilation and gas exchange variables were averaged over 1 min. Work of breathing (WOB) was calculated breath-by-breath as the area within the P_{es} -volume loop, multiplied by breathing frequency, converted into joules and averaged over 3 min. Every 3 min inspiratory

diaphragmatic, esophageal and gastric pressure–time products (PTP; $PTP_{di,in}$, $PTP_{es,in}$ and $PTP_{ga,in}$) as well as expiratory esophageal and gastric PTPs ($PTP_{es,ex}$ and $PTP_{ga,ex}$) were averaged over 10 consecutive breaths.

Unpaired t tests were used to assess differences in subjects' characteristics between runners and cyclists. For the comparison of twitch parameters and exercise response data between time-trials a linear mixed model was calculated with test (15TT and 30TT) and exercise modality (running and cycling) as main factors. To further account for differences among subjects a random intercept per subject was included. To test for supramaximal twitch stimulation a second linear mixed model was calculated with stimulator intensity as main factor. Post hoc pairwise comparisons were only made with 100 % stimulator output and Bonferroni correction was applied to correct for multiple comparisons. End-exercise parameters were compared between time-trials by use of the Wilcoxon signed rank test due to non-parametric distribution. Twitch reductions and exercise responses in normocapnic hyperpnoea were compared between runners and cyclists using unpaired t tests. Statistical analyses were performed with SPSS Statistics 19 (IBM Company, New York, NY, USA). All data are shown as mean \pm SD. The level of significance was set at $p < 0.05$ for all statistical comparisons.

Results

Runners covered a distance of 4.0 ± 0.2 and 7.7 ± 0.5 km (15TT and 30TT, respectively) at an average speed of 16.1 ± 0.8 and 15.3 ± 0.96 km h⁻¹ while cyclists covered a distance of 10.9 ± 0.9 and 20.2 ± 2.1 km (15TT and 30TT, respectively) at an average power output of 303 ± 31 and 279 ± 28 W.

Quadriceps muscle fatigue after running and cycling time-trials

Volitional quadriceps muscle strength, i.e. maximal voluntary quadriceps contraction, was significantly reduced after both 15TT and 30TT (main effect of time point, $p < 0.01$; Fig. 2). The reduction was not different between both time-trials ($p = 0.66$) and independent of the exercise modality (interaction test \times exercise modality, $p = 0.99$).

Quadriceps muscle contractility, i.e. potentiated Q_{tw} , was significantly reduced from baseline to post-exercise in both time-trials (both $p < 0.01$, Fig. 2). The reduction in Q_{tw} (ΔQ_{tw}) was significantly more pronounced in cyclists compared to runners (exercise modality, $p < 0.01$) while no significant difference between 15TT and 30TT was present (effect of test, $p = 0.34$) and no interaction was observed ($p = 0.06$). M-wave peak-to-peak amplitude was

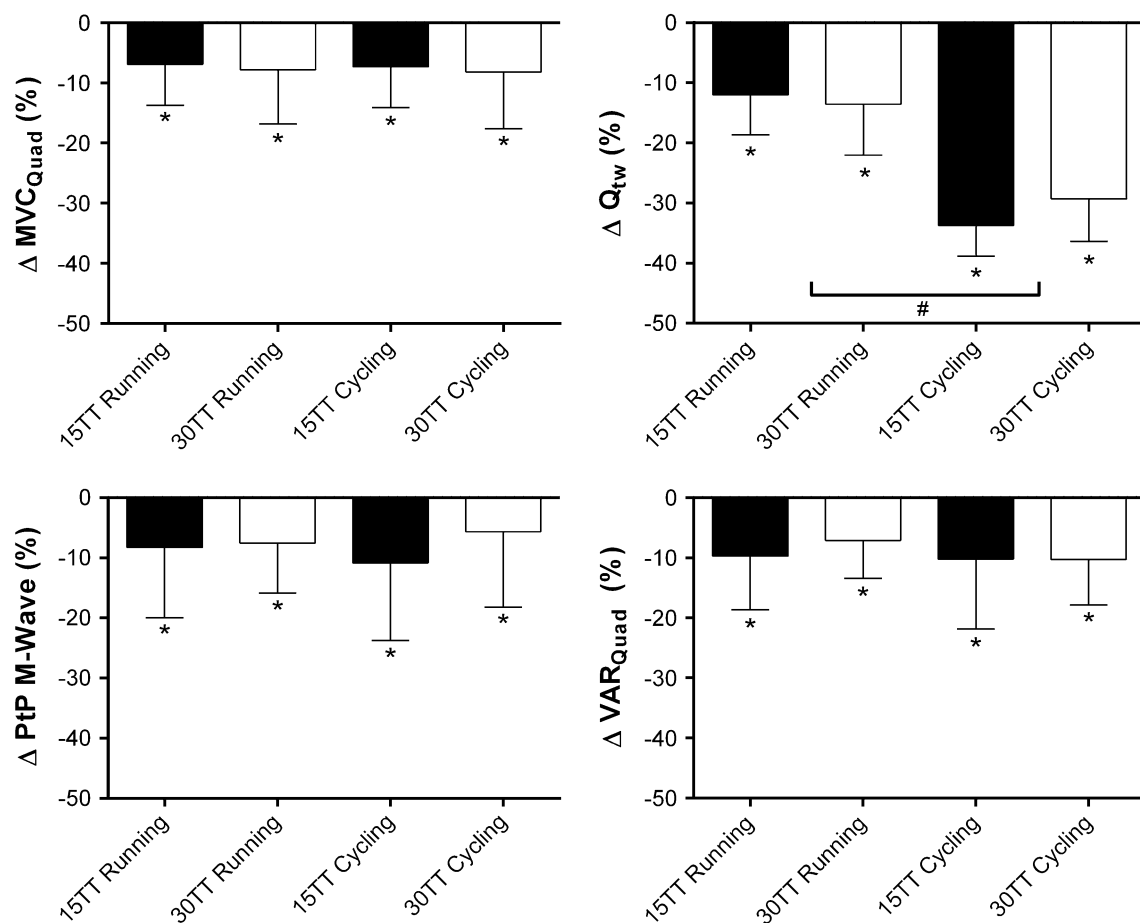


Fig. 2 Reduction in quadriceps muscle strength after 15 and 30 min time-trials (15TT, 30TT) in runners and cyclists. Values are mean \pm SD ($n = 12$). MVC_{Quad} , voluntary maximal contraction of the quadriceps; Q_{tw} , quadriceps twitch force; PtP M-Wave, peak-to-

peak amplitude of compound muscle action potentials; VAR_{Quad} , voluntary activation ratio of the quadriceps. *Significantly reduced compared to baseline, $p < 0.05$; #Significantly different between runners and cyclists, $p < 0.05$

significantly reduced after both time-trials with no effect of test ($p = 0.35$), exercise modality ($p = 0.93$) or their interaction ($p = 0.47$). Quadriceps voluntary activation ratio was similarly reduced after both time-trials irrespective of exercise modality (Fig. 2).

CV for potentiated Q_{tw} were similar at rest (15TT: 1.9 ± 1.3 %; 30TT: 2.4 ± 1.5 %) and after the time-trial (15TT: 4.2 ± 1.6 %; 30TT: 4.2 ± 1.8 %). The between-day CV of potentiated Q_{tw} was 4.4 ± 2.3 .

Diaphragm fatigue after running and cycling time-trials

Volitional diaphragm muscle strength, i.e. maximal voluntary diaphragm contractions, was significantly reduced after both time-trials indicated by a significant main effect of time point ($p < 0.01$, Fig. 3) without being different between 15TT and 30TT (main effect of test, $p = 0.62$) and exercise modality (interaction test \times exercise modality, $p = 0.86$).

Diaphragm muscle contractility, i.e. potentiated $P_{\text{di,tw}}$, was significantly impaired after both time-trials compared to resting values (both $p < 0.01$, Fig. 3). The decrease in $P_{\text{di,tw}}$ ($\Delta P_{\text{di,tw}}$) resulted from a significant reduction in both $P_{\text{es,tw}}$ and $P_{\text{ga,tw}}$ (all $p < 0.01$) and exceeded 10 % in all subjects in the 15TT and in 19/22 subjects in the 30TT. The level of $\Delta P_{\text{di,tw}}$ was significantly larger in the 15TT compared to the 30TT (effect of test, $p = 0.04$). The type of exercise (running or cycling) had no effect on the decrease in twitch pressures (no effect of exercise modality, $p = 0.78$ and interaction of test \times exercise modality, $p = 0.82$). The change in the ratio of $P_{\text{es,tw}}/P_{\text{ga,tw}}$ was not different between tests ($p = 0.29$) but tended to be different for running and cycling ($p = 0.06$, Fig. 3). The average P_{es} baseline immediately preceding each twitch was constant for all measurements, suggesting identical lung volumes before stimulations (15TT: before -4.6 ± 1.5 cmH₂O vs. after -4.8 ± 1.8 cmH₂O, $p = 0.52$; 30TT: before -4.8 ± 1.8 vs. after -5.1 ± 2.3 cmH₂O, $p = 0.37$). Diaphragm voluntary

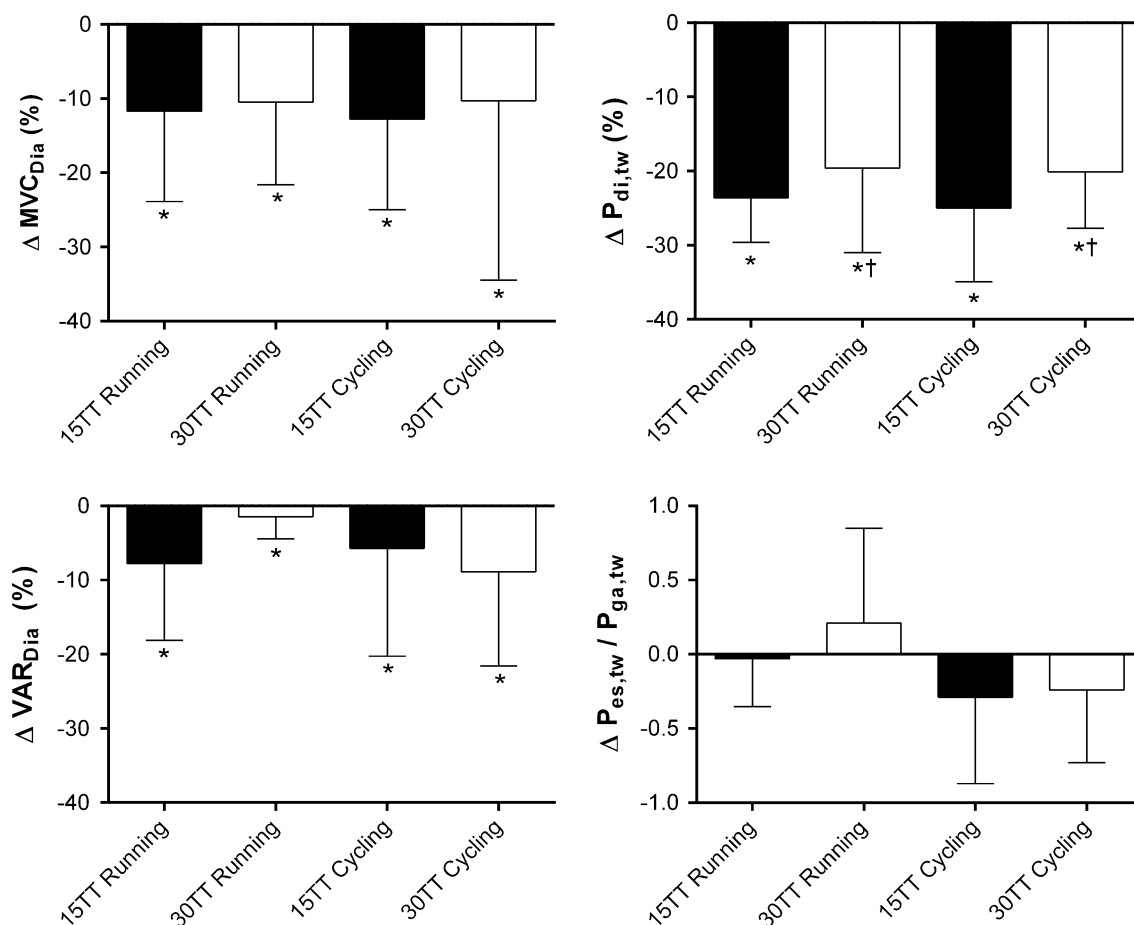


Fig. 3 Reduction in diaphragm muscle strength after 15 and 30 min time-trials (15TT, 30TT) in runners and cyclists. Values are mean \pm SD ($n = 12$). $P_{\text{di,tw}}$, transdiaphragmatic twitch pressure; $P_{\text{es,tw}}$, esophageal twitch pressure; $P_{\text{ga,tw}}$, gastric twitch pres-

sure; MVC_{Dia} , diaphragm voluntary maximal contraction; VAR_{Dia} , voluntary activation ratio of the diaphragm. *Significantly reduced compared to baseline, $p < 0.05$; †Significantly different from 15TT, $p < 0.05$

activation ratio was similarly reduced after both time-trials irrespective of exercise modality (Fig. 3).

Within-day coefficients of variation (CV) for potentiated $P_{\text{di,tw}}$ were similar at rest (15TT: 5.4 ± 2.9 %; 30TT: 5.6 ± 3.1 %) and after the time-trial (15TT: 6.8 ± 3.2 %; 30TT: 5.2 ± 2.1 %). The between-day CV of potentiated $P_{\text{di,tw}}$ was 4.0 ± 3.2 %.

Exercise response and respiratory muscle work during time-trial exercise

Average exercise responses for runners and cyclists during the 15TT and 30TT are given in Table 2 as well as in Fig. 4. $\dot{V}\text{O}_2$ was not different between runners and cyclists (main effect of exercise modality, $p = 0.25$) and significantly higher in the 15TT compared to the 30TT ($+4.4 \pm 4.5$ %, main effect of test, $p < 0.01$) with no interaction test \times exercise modality ($p = 0.38$). In addition, minute ventilation ($+6.4 \pm 7.3$ %) and inspiratory as well as

expiratory muscle work were higher in the 15TT indicated by a significant main effect of test (all $p < 0.001$) while no effect was observed for exercise modality and the interaction test \times exercise modality (Table 2). $\dot{V}\text{O}_2$ (runners and cyclists) rose to 93 ± 5 % $\dot{V}\text{O}_{2,\text{peak}}$ in the final 30 s of the 15TT and 88 ± 6 % $\dot{V}\text{O}_{2,\text{peak}}$ in the 30TT. Heart rate of the final 30 s reached 98 ± 4 % (15TT) and 99 ± 3 % (30TT) of the maximum heart rate obtained in the incremental test. Perception of respiratory and leg exertion reached maximal or near maximal levels (i.e. ≥ 9) at the end of the 15TT (in 18/22 subjects for respiratory exertion and in 19/22 for leg exertion) and 30TT (in 16/22 and 21/22 for respiratory and leg exertion, respectively).

Inspiratory muscle fatigue after isolated normocapnic hyperpnoea

Normocapnic hyperpnoea lasted for 7.8 ± 3.8 min in runners and 11.0 ± 9.0 min in cyclists ($p = 0.30$)

Table 2 Exercise response to time-trials

	15TT		30TT	
	Running	Cycling	Running	Cycling
HR (beats min ⁻¹)	174 ± 7	178 ± 6	169 ± 13	177 ± 8
Exercise intensity (% max)	84.1 ± 1.4	77.3 ± 4.1	79.9 ± 2.1	71.1 ± 4.0*, #
\dot{V}_E (l min ⁻¹)	125 ± 15	138 ± 20	120 ± 13	126 ± 16*
V_T (l)	2.77 ± 0.34	3.03 ± 0.40	2.64 ± 0.30	2.88 ± 0.35*
f_B (breaths min ⁻¹)	46.1 ± 7.0	46.1 ± 7.2	46.1 ± 6.2	44.6 ± 7.7
$PTP_{di,in}$ (cmH ₂ O s min ⁻¹)	737 ± 133	666 ± 229	658 ± 93	549 ± 164*
$PTP_{es,in}$ (cmH ₂ O s min ⁻¹)	444 ± 81	551 ± 142	404 ± 85	457 ± 89*
$PTP_{es,ex}$ (cmH ₂ O s min ⁻¹)	385 ± 135	373 ± 154	339 ± 109	309 ± 100*
$PTP_{ga,ex}$ (cmH ₂ O s min ⁻¹)	461 ± 133	416 ± 141	402 ± 90	293 ± 92*
WOB (joule min ⁻¹)	410 ± 137	516 ± 158	343 ± 109	384 ± 112*, ‡
$\dot{V}O_2$ (l min ⁻¹)	4.27 ± 0.30	4.51 ± 0.46	4.12 ± 0.38	4.27 ± 430*
$\dot{V}CO_2$ (l min ⁻¹)	4.29 ± 0.28	4.66 ± 0.55	4.01 ± 0.31	4.20 ± 0.50*, ‡
$\dot{V}O_2$ (% $\dot{V}O_{2,peak}$)	89.5 ± 3.5	89.5 ± 4.4	86.0 ± 3.8	84.6 ± 3.6*
$\dot{V}_E/\dot{V}O_2$	29.3 ± 2.7	30.3 ± 1.6	29.1 ± 2.8	29.3 ± 1.7
$\dot{V}_E/\dot{V}CO_2$	29.1 ± 2.8	29.5 ± 1.9	29.7 ± 2.4	30.0 ± 1.8
$P_{ET}CO_2$ (mmHg)	37.7 ± 3.2	37.6 ± 2.2	36.9 ± 2.9	37.2 ± 2.3
Respiratory exertion	9.0 ± 0.78	8.8 ± 1.2	8.7 ± 1.0	9.0 ± 0.8
Leg exertion	9.0 ± 1.0	9.3 ± 0.5	9.4 ± 0.7	9.1 ± 0.5
Blood lactate (mmol l ⁻¹)	7.95 ± 1.99	12.86 ± 1.99	5.92 ± 2.10	9.68 ± 1.74*, #

$P_{ET}CO_2$, end tidal CO₂ partial pressure for 15 and 30 min time-trials (15TT, 30TT). End-exercise values are given for perception of respiratory exertion, leg exertion and blood lactate concentration

Values are mean ± SD. HR heart rate, \dot{V}_E minute ventilation, V_T tidal volume, f_B breathing frequency, PTP pressure–time product, $PTP_{di,in}$ inspiratory diaphragmatic PTP, $PTP_{es,in}$ inspiratory esophageal PTP, $PTP_{es,ex}$ expiratory esophageal PTP, $PTP_{ga,ex}$ expiratory gastric PTP, WOB work of breathing, $\dot{V}O_2$ oxygen consumption; $\dot{V}CO_2$ carbon dioxide elimination

* Significantly different between 15TT vs. 30TT, $p < 0.05$

Significantly different between runners and cyclists, $p > 0.05$

‡ Interaction test × exercise modality, $p < 0.05$

at an average minute ventilation of 157 ± 12 and 149 ± 9 l min⁻¹ ($p = 0.08$) corresponding to 77 ± 4 % MVV in both groups ($p = 0.97$). Tidal volume of both groups averaged at 2.164 ± 0.385 l (corresponding to ~35 % of vital capacity) and breathing frequency was 74 ± 12 breaths min⁻¹ without being different between runners and cyclists. During the course of normocapnic hyperpnoea, tidal volume decreased significantly while breathing frequency increased (tidal volume: from 2.588 ± 0.518 to 1.796 ± 0.415 l; breathing frequency: from 63 ± 12 , to 85 ± 16 breaths min⁻¹) independent of exercise modality. Normocapnia was achieved in all subjects throughout normocapnic hyperpnoea with an average end-tidal CO₂ partial pressure of 39.2 ± 1.2 mmHg.

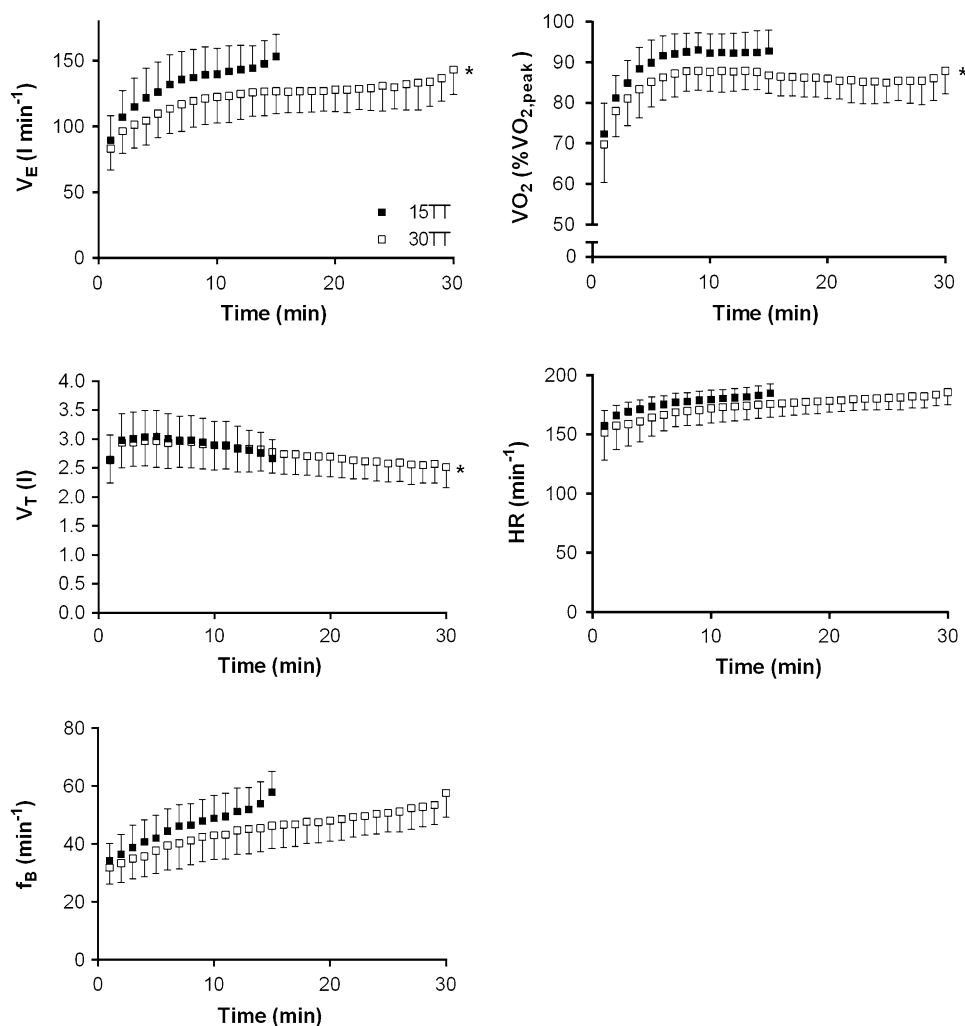
After normocapnic hyperpnoea, $P_{m,tw}$ was significantly reduced by 35.8 ± 11.0 %. Data of one subject had to be excluded due to the inability to produce reliable $P_{m,tw}$ measurements after normocapnic hyperpnoea. The average reduction in $P_{m,tw}$ for runners (37.1 ± 8.4 %, $n = 11$)

and cyclists (34.4 ± 13.5 %, $n = 10$) were not significantly different ($p = 0.60$). Within-day CVs for $P_{m,tw}$ were 6.1 ± 2.5 % before and 7.8 ± 3.9 % after normocapnic hyperpnoea.

Discussion

The present study sought to investigate differences in the level of fatigue in locomotor and diaphragm muscles during self-paced exercise of different durations (15 vs. 30 min) and between different exercise modalities (running and cycling). Present findings obtained in endurance trained runners and cyclists (in their respective discipline) suggest that both quadriceps and diaphragm muscle fatigue is present after time-trials but quadriceps muscle fatigue is substantially more pronounced in cyclists compared to runners (independent of exercise duration/intensity) while diaphragm fatigue is more pronounced in the

Fig. 4 Development of ventilatory variables, relative oxygen consumption and heart rate during 15 and 30 min time-trials (15TT, 30TT). Values are mean \pm SD ($n = 22$); \dot{V}_E minute ventilation; V_T tidal volume, f_B breathing frequency, $\dot{V}O_2$ oxygen consumption, HR heart rate ($n = 21$)



shorter, more intense time-trial (independent of exercise modality). In addition, no difference in time to exhaustion and in the rate of fatigue development was evident between runners and cyclists performing isolated normocapnic hyperpnoea.

Quadriceps muscle fatigue after running and cycling time-trials of different durations

Both time-trials (15 and 30 min) induced significant reductions in volitional maximal quadriceps muscle force. This reduction is likely to be the result of both a reduction in muscle contractility and the ability to voluntarily drive the muscle as both Q_{tw} amplitude and quadriceps voluntary activation ratio were significantly decreased after time-trials (Fig. 2). The fact that M-Wave amplitude was also reduced in both time-trials (Fig. 2), indicates that peripheral fatigue (ΔQ_{tw}) may have included not only contractile failure but possibly also alterations in the electrical propagation of the action-potential across the neuromuscular

junction and along the sarcolemma and t-tubules (i.e. reduced membrane excitability).

The reductions in maximal voluntary quadriceps contraction (7 %) and Q_{tw} (13 %) after running time-trials are in line with other studies investigating quadriceps muscle fatigue in tests of similar duration/intensity (Nummela et al. 2008). Similarly, reductions in maximal voluntary quadriceps contraction after cycling time-trials are in the range of those found in other studies using cycling tasks of approximately the same duration (Bentley et al. 2000; Lepers et al. 2002). Reductions in Q_{tw} in cyclists were, however, slightly larger than those in the latter studies and are more consistent with those seen in somewhat shorter time-trials [e.g. (Amann et al. 2006)]. To the best of our knowledge, this is the first time that locomotor muscle fatigue was systematically investigated after self-paced exercise of different durations/intensities in the same group of individuals. Interestingly, peripheral quadriceps muscle fatigue was not different between the 15 and 30TTs. These results may be viewed in support of the idea of afferent feedback

interacting at the spinal and/or supraspinal level possibly to avoid the development of fatigue beyond a certain functional limit (Amann and Secher 2010; Racinais et al. 2007). Alternatively, fatigue was suggested to be the consequence of a metabolic challenge mainly to type 2 fibers and to develop rather early during exercise which would then explain similar levels of contractile fatigue (Decorte et al. 2012; Sargeant 2007).

However, Q_{tw} was substantially more impaired in cyclists than in runners after time-trials in the present study. Both methodological and physiological mechanisms may explain these findings. First, one has to consider whether the quadriceps is the ‘right’ muscle to compare the occurrence of fatigue in locomotor muscles between running and cycling. In fact, there is evidence showing that EMG activity of the quadriceps muscle does not increase linearly with the increasing mechanical power output for running but does so during cycling (Bijker et al. 2002). For the calf muscles on the other hand, a linear relation between EMG activity and mechanical power was detected in both running and cycling exercise which could imply that this muscle group would have been better comparable. However, when Martin and co-workers (2010) measured muscle contractility in both the knee extensors and plantar flexors at the same time, no difference in the amount of reduction in twitch force was present. Furthermore, when looking at running exercise of comparable duration to our study, reductions in twitch force of the calf muscle [i.e. ~16 % (Girard et al. 2012)] were of similar magnitude as those found for the quadriceps muscle here (~13 %). Hence, we believe that the quadriceps muscle represents a valid model for comparing the occurrence of locomotor muscle fatigue in running and cycling. Second, since runners and cyclists only performed in their respective sports, one has to consider that two different populations were investigated which per se could have affected the level of contractile fatigue. The present study design was introduced to minimize a potential bias of running/cycling experience on time-trial performance but clearly limits what can be concluded in terms of the mechanism responsible for the finding of augmented levels of contractile fatigue in cyclists. Despite of these limitations, it is important also to consider the type of muscle contraction specific to running and cycling. The quadriceps muscle is exposed to repeated stretch–shortening cycles (i.e. eccentric and concentric contractions) during running while cycling predominantly involves concentric contractions (Bijker et al. 2002). Owing to eccentric contractions in running, one would expect both metabolic and mechanical stress to contribute to the loss in Q_{tw} while mechanical stress is likely less prominent and metabolic stress more pronounced when cycling. There is indeed some indication that metabolic load on the quadriceps was higher in cyclists than in runners as relative whole-body

$\dot{V}O_2$ was not different between exercise modalities but blood lactate concentrations at the end of exercise was higher in cyclists (Table 2). In fact, both metabolic and mechanical stress can impair contractile function and thus exercise performance (Amann and Dempsey 2008; Marcora et al. 2008). Contractile impairment, however, was reported to differ between concentric and eccentric contractions both in terms of magnitude and timing (Smith and Newham 2007). The latter authors reported that contractile fatigue was less pronounced after eccentric compared to concentric contractions and that peak reduction after eccentric contractions occurs ~60 min after task cessation while in concentric exercise peak reduction occurred ~20 min into recovery. Thus, the time point when Q_{tw} was reassessed (i.e. 10 min after completion of time-trials) after time-trials might explain some of the observed differences between runners and cyclists in the present study. Interestingly, despite smaller levels of peripheral muscle fatigue in runners, similar reductions in maximal voluntary quadriceps contractions were detected in presence of a similar drop in voluntary activation ratio (Fig. 2). Unfortunately, the present study does not allow for a conclusive discussion. Apart from methodological concerns regarding the sensitivity of the superimposed twitch technique (Gandevia 2001), one could also hypothesize that this apparent discrepancy points towards effects of muscle damage (due to eccentric contractions in running) which act beyond the excitation–contraction coupling system and are thus not detected by the assessment of Q_{tw} (Iguchi and Shields 2010). This clearly needs further investigations.

Diaphragm muscle fatigue after running and cycling time-trials of different durations

The average intensities of both self-paced exercise tests were 90 % (15TT) and 85 % $\dot{V}O_{2,peak}$ (30TT), i.e. they were performed at and above the intensity (i.e. ≥ 85 % $\dot{V}O_{2,peak}$) reported to be required for diaphragm fatigue to occur (Johnson et al. 1993). The degree of diaphragm fatigue that subjects developed in the two time-trials compared well to that observed after exhaustive constant-load exercise (Babcock et al. 1995a, b, 2002; Johnson et al. 1993; Mador et al. 1993; Taylor et al. 2006; Verges et al. 2007, 2006a, b; Walker et al. 2011; Wuthrich et al. 2013). The one study that assessed diaphragm fatigue after constant-load exercise at 90–95 % and 80–85 % $\dot{V}O_{2,peak}$ unfortunately included a different number of subjects with a large variation in fitness level and the actual exercise intensity ranged from 60 % to over 110 % $\dot{V}O_{2,peak}$ in the two trials such that a direct comparison is not possible (Johnson et al. 1993). Thus, the hypothesis that more intense cycling would cause larger degrees of diaphragm fatigue was proposed but has not been investigated systematically. Present findings

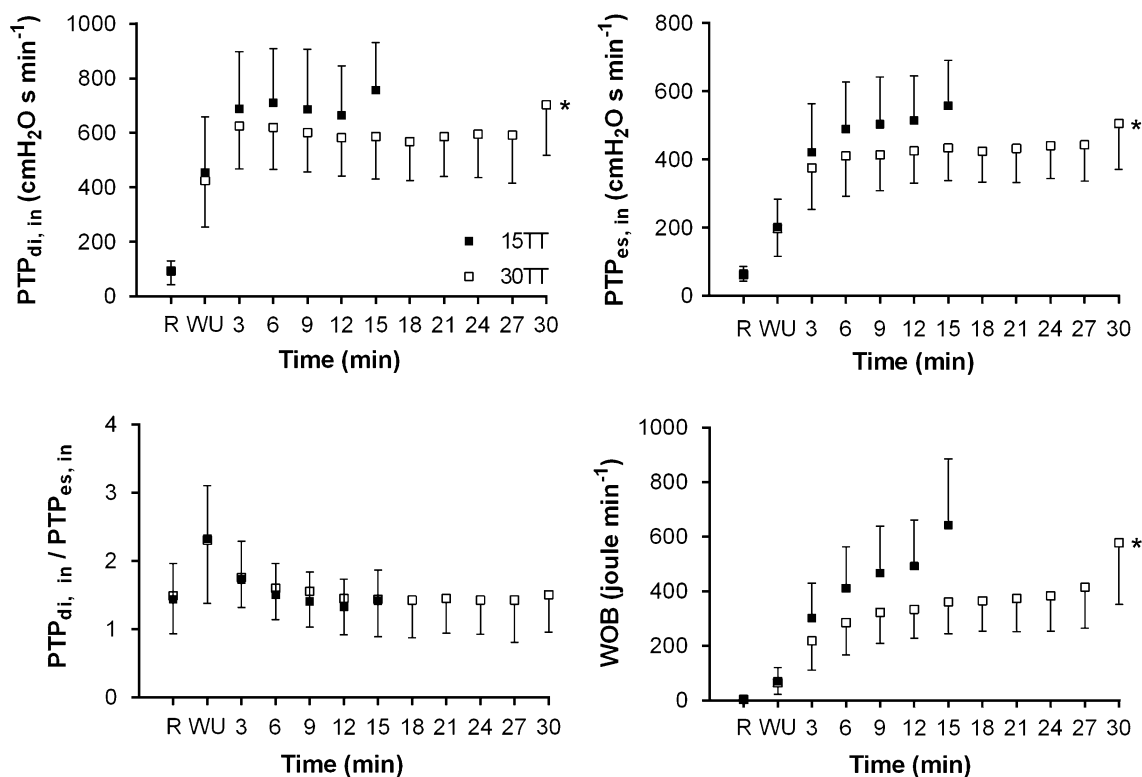


Fig. 5 Variables of respiratory muscle work during 15 and 30 min time-trials (15TT, 30TT). Values are mean \pm SD ($n = 22$). R rest, WU warm up, PTP_{di,in} inspiratory transdiaphragmatic pressure–time

product, PTP_{es,in} inspiratory esophageal pressure–time product, WOB work of breathing

suggest substantially more pronounced levels of diaphragm fatigue after the shorter more intense 15TT ($\Delta P_{di,tw}$ 24 %) than after the longer 30TT ($\Delta P_{di,tw}$ 20 %)—a finding which was anticipated given the relationship between exercise intensity and the level of diaphragm fatigue observed by Johnson and co-workers (1993). Several factors may have contributed to the observed difference in diaphragm fatigue while total respiratory muscle work that accumulated during the entire exercise duration can be excluded, i.e. the sum of WOB was approximately 1.5 times larger in the 30TT where fatigue was smaller. However, when considering respiratory muscle work on a per minute basis, we found significantly higher levels for the shorter, more intense 15TT (Table 2), i.e. the higher exercise intensity of the 15TT not only induced a significantly higher average ventilation (+6.4 %) but also an even more pronounced increase in respiratory muscle work per minute (+16 % in PTP_{di,in}, +15 % in PTP_{es,in} and +27 % in WOB; Fig. 5).

Of note is that higher exercise intensity not only increases respiratory muscle work but may also affect blood flow distribution to all working muscles. While increased respiratory muscle work was shown to compromise leg blood flow (Harms et al. 1997), two recent studies reported that blood flow to intercostal muscles may actually

be compromised at high ventilatory levels during whole-body exercise (Henderson et al. 2012; Vogiatzis et al. 2009). The latter studies showed that intercostal blood flow reaches a peak around 80 % maximal cycling power output in an incremental test. In the 15TT of the present study, average relative running velocity (84 % maximal running velocity) and cycling power output (80 % maximal cycling power output) were in fact at or above this threshold suggesting the potential for blood flow restrictions to respiratory muscles in contrast to the 30TT, where relative running velocity (80 % maximal running velocity) and cycling power output (71 % maximal cycling power output) were at or clearly below this threshold suggesting no compromise of the respiratory muscle blood flow. The potentially larger mismatch between oxygen demand and supply in the inspiratory muscles along with higher respiratory muscle work during the more intense 15TT may have led to an increased accumulation of metabolites known to impair the function of the contractile apparatus thus explaining in part why diaphragm fatigue was more pronounced in the shorter 15TTs (Allen et al. 2008).

Contrary to our original hypothesis, similar levels of inspiratory muscle fatigue were observed for runners and cyclists. We hypothesized that diaphragm fatigue would be

more pronounced in runners due to the postural demand associated with running. We based our hypothesis mainly on findings by Hodges and Gandevia (2000) who reported tonic and phasic diaphragm activity during postural challenge superimposed on breathing. We suggested that this postural demand in concert with severe ventilatory work might place a greater load on the diaphragm in runners compared to cyclists. This is supported by an immediate increase in P_{ga} at the start of exercise which was consistently seen in runners but not in cyclists (data not shown). This increase in intra-abdominal pressure is thought to help improving the stiffness and stability of the spine when the maintenance of posture is challenged but may also increase the load on the working diaphragm (Henke et al. 1988; Hodges et al. 2005). This increase in P_{ga} also increased $PTP_{ga,in}$, thus explaining why the ratio of $PTP_{es,in}/PTP_{di,in}$ was higher in runners than cyclists (data not shown). These observations suggest a somewhat higher postural demand in runners which does, however, not seem to exert a substantial effect on the development of inspiratory muscle fatigue.

Although inspiratory muscle fatigue was not different between exercise modalities, the contribution of the diaphragm and of rib cage muscles might have differed between running and cycling since in runners, the ratio of $P_{es,tw}/P_{ga,tw}$ (Similowski et al. 1998) remained unchanged (15TT) or even slightly increased (30TT) while in cyclists it was reduced in both time-trials with the effect of exercise modality showing a tendency to statistical significance ($p = 0.06$). Together with significantly reduced $P_{di,tw}$ and $P_{ga,tw}$ in both exercise modalities, this may be interpreted to mean that running time-trials induced predominantly diaphragm fatigue while cycling induced more global inspiratory muscle fatigue (Similowski et al. 1998). The significance of this potential difference was then tested in volitional normocapnic hyperpnoea where no difference in postural demand was present for subjects trained in the different exercise modalities.

In contrast to our hypothesis task failure in volitional normocapnic hyperpnoea occurred slightly but not significantly earlier in runners than in cyclists. Runners performed at somewhat higher absolute ventilation but at the same relative ventilation as cyclists (i.e. 77 % MVV) and inspiratory muscles were similarly fatigued in runners and cyclists indicated by a 36 % reduction in $P_{m,tw}$. Also, runners and cyclists showed similar rates of fatigue development (i.e. the reduction in $P_{m,tw}$ divided by the duration of normocapnic hyperpnoea, data not shown) which further highlights the finding of similar fatigue resistance for both groups.

Collectively, results from normocapnic hyperpnoea tests confirm findings during whole-body exercise where the magnitude of inspiratory muscle fatigue was similar in runners and cyclists. Together, one could postulate that

ventilatory demand during whole-body exercise substantially exceeds that of posture with the latter consequently not affecting the development of fatigue to a significant degree—thereby also not inducing specific training adaptations. This, however, requires further investigation but is supported by the finding that posture-related diaphragm activity decreases when ventilatory demand increases (Hodges et al. 2001).

Methodological considerations and limitations

Influence of end-expiratory lung volume on the assessment of diaphragm fatigue

Constant lung volumes before application of magnetic stimulations are crucial to achieve reproducible twitch measurements since an inverse linear relationship exists between $P_{di,tw}$ and lung volume (Hamnegard et al. 1995; Walker et al. 2011). Thus, lung volumes were closely monitored in the present study by measurement of P_{es} before each stimulation. Baseline P_{es} was similar within a sequence of twitches and also before and after exercise. Thus, altered lung volumes are unlikely to have biased our outcomes but lung volumes could not be monitored during $P_{m,tw}$ measurements. Also, similar CVs of $P_{m,tw}$ and $P_{es,tw}$ suggest that lung volumes were constant also during $P_{m,tw}$ assessments.

M-Wave during assessment of diaphragm fatigue

EMG responses to magnetic stimulation were not assessed for inspiratory muscles. This opens the possibility of reduced excitability of the muscular membrane resulting from electrolytic disturbances after exercise which would lead to an overestimation of contractile fatigue (Fowles et al. 2002). However, such changes were not commonly seen in studies assessing diaphragm fatigue and EMG concomitantly in our laboratory or those of others (Guenette et al. 2010; Johnson et al. 1993; Verges et al. 2006a). Hence, an overestimation of contractile diaphragm fatigue in the present study is unlikely, although it cannot be completely ruled out.

Selection of investigated population

In the present study, only experienced endurance athletes were included performing in their respective exercise modalities in order to guarantee replication of a maximal effort as well as optimal pacing in both the 15TT and 30TT. This is a prerequisite when investigating potentially limiting processes within the human body and was likely to be achieved here as indicated by several findings. Perception of respiratory and leg exertion increased to ≥ 9 (out

of a maximum of 10) by the end of the test in most of the subjects and heart rate of the final 30 s of both time-trials was at 98–99 % of the maximal heart rate achieved in the incremental test. Also, average $\dot{V}O_2$ of the final 30 s rose to approximately 93 % (15TT) and 88 % of $\dot{V}O_{2,peak}$ (30TT) which is in line with other studies where maximal time-trial exercise or exhaustive constant-load tests were performed (Amann et al. 2009; Johnson et al. 1993). These findings strongly suggest that subjects achieved a truly maximal effort in both time-trials. Yet, it remains speculative how these results may be transferred to other populations such as healthy subjects of different sex and age or patients. Existing studies on the development of quadriceps muscle fatigue at the limit of tolerance suggest that levels of fatigue are remarkably similar between young and elderly individuals as well as patients suffering from chronic obstructive pulmonary disease (Amann 2011; Mador et al. 2000a, b, 2001; Saey et al. 2005) while findings for diaphragm fatigue seem to be more variable when considering healthy subjects of different fitness and age or patients suffering from chronic obstructive pulmonary disease (Babcock et al. 1996; Mador et al. 2000a; Polkey et al. 1995).

Conclusion

The present study provides evidence that alterations in contractility of the quadriceps muscle after intense self-paced whole-body exercise are distinctly different for running and cycling while these alterations are very similar in the two exercise intensities of a single exercise modality. The level of inspiratory muscle fatigue is, however, independent of the exercise modality and related more to exercise intensity and the associated respiratory muscle work. Possibly, the postural demand placed upon the diaphragm during running resulted in a larger contribution of diaphragmatic fatigue to total inspiratory muscle fatigue compared to cycling which did, however, not lead to greater fatigue resistance during volitional hyperpnoea in runners compared to cyclists.

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Conflict of interest The authors declare that no conflict of interest exists.

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